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(54) Capillary zone electrophoresis cell system.

(57) A method and apparatus for increasing detector sensitivity in a Capillary Zone Electrophoresis detector is disclosed. A narrow bore capillary (10) includes an injection end (12) and an output end (30). Each end is placed in a reservoir (14,28) containing a buffer solution (15) and a sample of solute (16). The solute (16) comprises at least one unknown constituent component (17). An electric field is imposed across the buffer solution (5) and solute (16) in the capillary (10) by a power supply (22) coupled to leads (20,24) and electrodes (18,26). A source of ultraviolet light (38) illuminates a cell (32) within the capillary (10) and a sensor (42) measures the absorbance of radiation by the solute (16) to detect the constituent components(17).

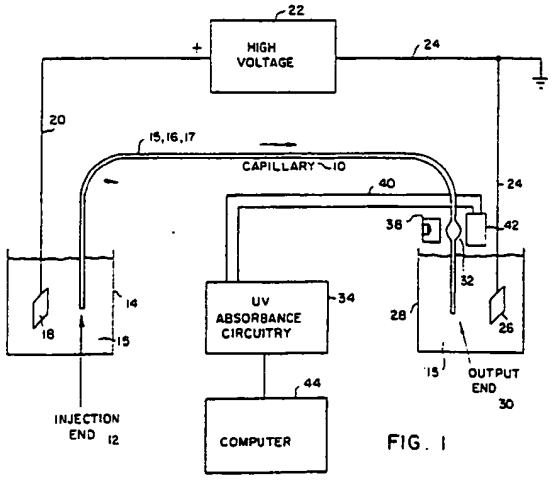

FIG. 1

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detection circuit of the narrow bore capillary. The Capillary Zone Electrophoresis Cell System is capable of sensing injected substances at the remarkable sensitivity threshold of one part per million concentration. This achievement has been accomplished with an system that is economical, easy to use, and highly reliable.

A tiny capillary with an inside diameter about as thin as a hair conducts a carrier fluid from an injection end to an output end. Both ends of the capillary are placed in reservoirs which also contain the carrier fluid, which is usually called a buffer solution. At the start of the separation, micro-grams of an unknown substance are inserted into the injection end of the capillary. An electric field is then imposed across the capillary. Due to the electrical field, charged component parts of the unknown sample move from one end of the capillary to the other end. During the last stages of their migration, the unspecified constituents of the sample enter an egg-shaped cell. While in the cell, ultraviolet light is directed toward and through the cell. Part of the light is absorbed by the biological sample. The level of absorbtion is measured by a detector, and, based upon comprehensive and widely-available knowledge which indicates which types of substances absorb various amounts of light, an analysis of the sample is obtained.

The Applicant's Capillary Zone Electrophoresis Cell System is a diagnostic and measurement device that offers an unprecedented sensitivity in detecting the constituent factors of microscopic samples. The apparatus claimed in this patent application provides an effective, efficient, and powerful tool that will enable engineers and scientists in the medical instrumentation industry to construct analysis equipment that will revolutionize the critically important field of biotechnology.

An appreciation of other aims and objectives of the present invention and a more complete and comprehensive understanding of this invention may be achieved by studying the following description of a preferred embodiment and by referring to the accompanying drawings.

Figure 1 is a schematic diagram that illustrates a Capillary Zone Electrophoresis Cell System.

Figure 2 is a side view of the cell showing the electric field and isopotential lines.

Figure 3 is a side view of the cell that also reveals bands of solute borne by the buffer solution passing through a beam of ultraviolet radiation.

Figure 4 is a schematic comparison of photon flux and path length through three different size capillary cross-sections.

Figure 5 is a comparison of normalized detected bands showing the reduction in noise achieved by the using successively larger cell diameter sizes shown in cross-section in Figure 4.

Figure 6 is a graph which plots detector response against the concentration of a detected solute and which illustrates the desirable linear characteristic of the present invention.

Fife 7 ia a graph which reveals the sensitivity of the Capillary Zone Electrophoresis Cell System. The sharp drop in the curve from left to right shows the abrupt change in absorbance of a protein equivalent present in the cell at a concentration level of only one part per million.

Figure 8 is a schematic side view of a glass lathe that is employed to fabricate the novel CZE cell.

Figure 1 is a schematic illustration of a Capillary Zone Electrophoresis (CZE) Cell System which includes a narrow bore capillary 10 having an injection end 12 located in an injection reservoir 14. The capillary 10 is fabricated from fused silica and measures about 50 microns across its inside diameter, although different-sized micro-bore capillaries are envisioned by the inventor. Injection reservoir 14 supplies the capillary 10 with an electrolytic aqueous medium of constant isocratic solution, such as a simple salt or buffer solution 15. A sample 16 having unknown constituent parts 17 is inserted into the injection end 12 by momentarily dipping the end 12 into a sample vial (not shown) and by drawing a small amount of sample 16 into the capillary 10 by the application of voltage or pressure. A high voltage power supply 22 is connected to a positive electrode 18 which is situated in injection reservoir 14. A ground lead 24 connects the power supply 22 to a negative electrode 26, which is similarly located in an output reservoir 28. An output end 30 of capillary 10 is placed in output reservoir 28.

A novel cell 32 is formed within the capillary 10 and is illuminated by a source of ultraviolet (UV) light 38. Throughout the specification and claims, the terms "containment means", "dilated zone", and "cellular containment means" refer to the cell as pictured in the drawings and as described by the specification. A detector 42 receives light from source 38 after it passes through the cell 32 and is absorbed by components 17 of any solutes 16 that are carried in the buffer solution 15. The detector 42 is connected to ultraviolet absorbance circuitry 34 by leads 40. Measurements may be interpreted and displayed by a computer 44 or by a strip-chart recorder (not shown) which receives signals from the absorbance circuitry 34.

Figure 2 reveals one embodiment of the novel cell 32 in great detail. The smallest diameter of the cell 32 is represented by the distance across the pairs of arrows labeled "46". In the best mode of the invention, this dimension is fifty microns and matches the inside diameter of the narrow bore

performed after the signals are amplified above the circuit noise level to combine the sensitivity of curve 96 with the linearity of curve 94. The extended linearity of both curves 94 and 96 is far greater than that attainable with previous methods that utilize detection on conventional capillaries without the novel cell 32 claimed in this application, because the novel cell 32 helps to eliminate stray light that would otherwise skirt the sides of a much smaller target and that would not pass through the solute 16.

Figure 7 reveals the striking results of placing less than one part of average protein equivalent in a million parts of buffer solution and passing the mixture through the detector. The left portion of the curve 98 is a detector output showing a first level of absorbance of pure buffer solution. When the one part per million of protein equivalent passes into the CZE cell, the absorbance increases dramatically, as indicated by the reduced detector output shown by the right portion of the curve, labeled 100. This one drawing vividly expresses the power of the present invention to sense complex biological substances in minute concentrations.

Figure 8 is a schematic side view of a cell lathe 102 that is employed to fabricate the novel CZE cell 32. The lathe 102 includes a base 104 that anchors motor 106 which is connected by a drive shaft 107, supported by bearing 110, to drive gears 108. Drive gears 108, in turn, are coupled to driven gears 112 and 114, which are mounted on rotating hollow shafts 113 supported by bearings 116. Chucks 118 are mounted on hollow shafts 113 and provide support for capillary 120 at two points. Capillary 120 is bolstered by support 122 and is held by a pair of chucks 118. The operator of the lathe 102 views a central section of capillary 126 that is heated by a small flame through microscope 124. Micro-blowing methods that scaled-down versions of techniques which are well-known to persons ordinarily skilled in the glass fabrication art were employed to manufacture the CZE cells 32. The capillary 120 is first sealed off at one end, and then a syringe 128 is used at the other end to pressurize the volume within it to ten to twenty percent over-pressure. A high quality cell 32 may be formed by rotating the capillary 10 in a 1/16" gas flame for three or four seconds.

Operation of the CZE System

The motivating force which drives the separation of analytes in the present invention is an electric field which is imposed across the capillary. A potential difference of thirty to fifty kilovolts causes substances borne by the carrier to move from one end of the tube to another, but the movement itself has two components. The more apparent and more readily understood of the two is electrophoretic migration. Electrophoresis refers to the process of charged molecules moving toward an oppositely charged electrode due to the simple electrical attraction of dissimilar charges. The second reason for movement of materials through the CZE system is a phenomenon called electro-osmotic flow. Whenever a liquid is placed in a glass tube, molecules in the liquid that have a negative charge tend to stick to the walls of the tube. Positive charges do not behave this way. This preferential sticking or adsorbtion of negatively charged molecules attracts a thin layer of positively charged molecules all along the inner walls of the capillary. The electric field pulls these positive molecules or ions toward the negative electrode at the output end of the capillary. The positive ions drag other molecules along with them, even those having a neutral or negative charge. The result is a flow of all kinds of differently charged solute moving toward the output end of the capillary. Electro-osmotic flow provides the pumping force which moves molecules of all charges toward one end of the system.

The strength of the electro-osmotic force that generates the bulk flow in the capillary is directly proportional to the applied electric field. Within the CZE cell, the field is lower than the strength of the field in the capillary due to the larger cross-section of conductive fluid within the cell. Since the field is inversely proportional to the fluid area, the field is only one sixteenth as strong within a cell that has a diameter that is four times greater than that of the capillary. In this cell, the osmotic force at the capillary wall within the cell is also sixteen times weaker than the osmotic force at the walls in the rest of the capillary. If the flow were constant, this force would be too small and would result in a laminar flow in which solute at the edge of the cell would lag behind solute in the center of the cell. This uneven transport would cause undesirable spreading of the peaks and would degrade the performance of the CZE system. As a direct consequence of the design of the present invention, this problem does not occur. As this osmotic bulk-flow force drops in the cell, the flow velocity in the cell also drops in proportion to the cross-sectional area of the cell. When the bulk flow slows down in the cell, the forces that propel it fall concomitantly. The extremely beneficial result is a balance of forces on the bands of analyte and an absence of forces that would tend to distort the bands and broaden the peaks.

Design Considerations

of said capillary (10); both of said injection and said output reservoirs (14,28) being capable of holding said buffer solution (15) and said substance (16); and a power supply (22) coupled to a first electrode (18) and to a second electrode (26).

4. An apparatus according to claims 2 or 3 in which said cell (32) has a cell length (57) that is approximately equal to the inside diameter (48) of said cell (32).

5. An apparatus according to any of claims 2 to 4 in which said cell (32) has a curved portion (50) having a longitudinal projection (56) of said curved portion (50) that is within an order of magnitude of an outside diameter (48) of said cell (32).

6. An apparatus according to any preceding claim, wherein said capillary (10) is ultra-violet transparent, said cell (32) is integrally formed with said capillary, said electric field generator (18,20,22,24,26) acts as an electrophoretic means for moving said substance (16) through the capillary, said radiation source (38) illuminates a central part of said cell, and the apparatus further comprises amplification means (34,44) responsive to said radiation sensor (34,40,42) for displaying an electronic output that indicates the concentration of said substance in the cell.

7. An apparatus as claimed in any preceding claim, in which said radiation sensor (42) is a photodiode.

8. An apparatus as claimed in any of claims 1 to 6, radiation sensor (42) is a photomultiplier tube.

9. An apparatus as claimed in any of claims 1 to 6, in which said radiation sensor (42) senses an absorbtion of ultra-violet light by said substance (16).

10. An apparatus as claimed in any of claims 1 to 6, in which said radiation sensor (42) senses a fluorescence light emitted by said substance (16).

11. A method of identifying an unknown sample comprising: providing a tubular vessel (10) capable of holding a transport medium (15) and a substance (16) having at least one constituent component (17); imposing an electric field across said tubular vessel (10) to move said transport medium (15) and said substance (16) through said vessel (10); directing a beam of radiation toward said substance (16) while it passes through a cell (32) within said vessel (10); and measuring a level of radiation absorbed by said substance (16) to identify said constituent component (17).

12. A method of fabricating a cell (32) comprising a part of an apparatus according to any preceding claim, the method comprising the steps of heating a small portion of said capillary (10); and pressurizing said capillary to dilate said capillary to form said cell (32).

FIG. 2

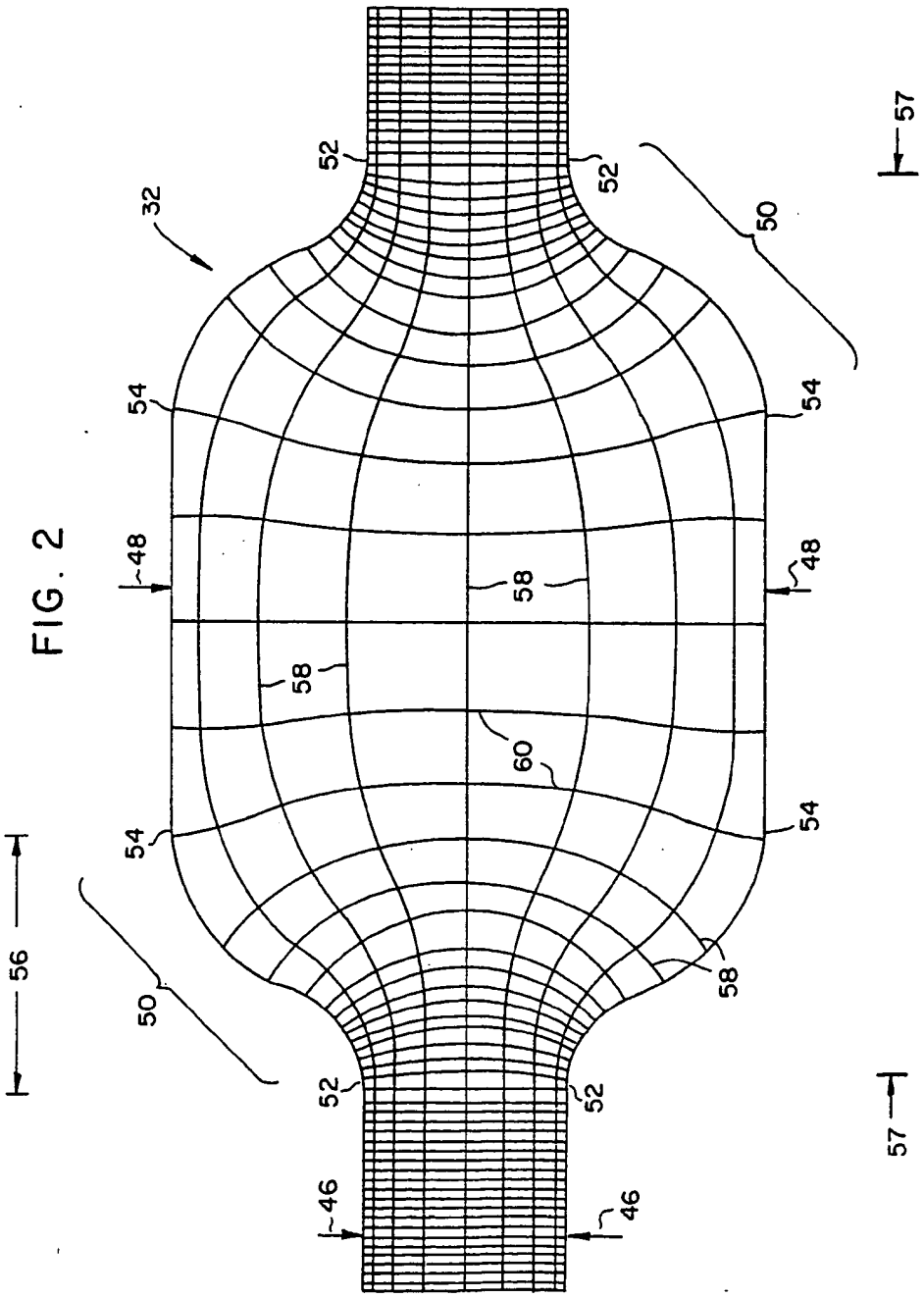


FIG. 4A

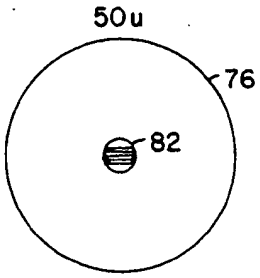


FIG. 4B

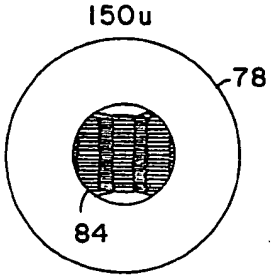


FIG. 4C

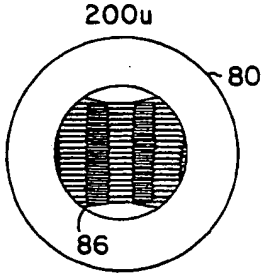
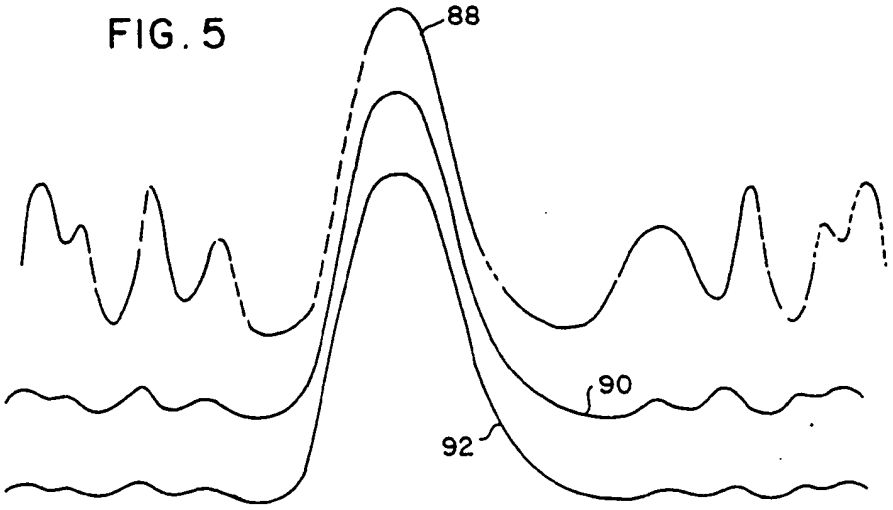


FIG. 5



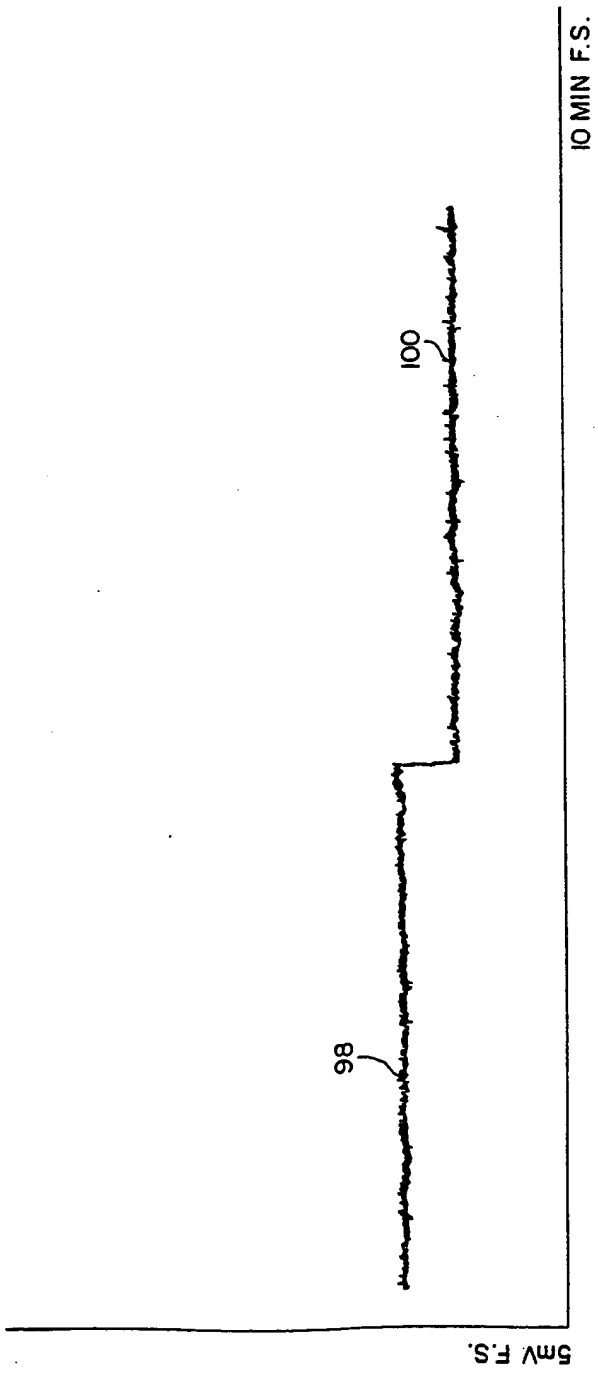


FIG. 7

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 30 2093

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	APPLIED SPECTROSCOPY, vol. 43, no. 2, February 1989, pages 196-201, Society for Applied Spectroscopy, Frederick, MD, US; M. YU et al.: "Attomole amino acid analysis: Capillary-zone electrophoresis with laser-based thermo-optical detection" * Pages 196-201 *	1	G 01 N 27/447
A	EP-A-0 216 600 (R. ZARE) * Figures 3,4 *	1	
A	FR-A-2 558 262 (E. LEROY) * Figure 3 *	1	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

G 01 N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14-06-1990	DUCHATELLIER M.A.

CATEGORY OF CITED DOCUMENTS

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